

Updates from MHDL

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Today's Updates:

♦Virus Culture at MHDL ♦Audioconferences ♦Annual PFGE summary ♦Influenza update ♦SurvNet Report

Virus Culture Continues ~ 60 Years at MHDL

Dr. Henry J. Wisniewski set up one of three municipal diagnostic virology laboratories in the US (N.Y. & L.A) at the Milwaukee Health Department in 1953 to address influenza and polio. Techniques were refined by Dr. Gerald Sedmak who originated our labor-savings multi-well technique, which complements our molecular testing for public health clinics, investigations and surveillance of environmental and clinical as well as reference work.

Multi-well Virus Culture and Isolation at MHD Laboratory

The Milwaukee Health Department Laboratory (MHDL) has used 24-well plates for virus isolation for over 20 years. This simple but innovative technique has several advantages over conventional tube and flask cultures. During passage of stock flasks of various cell lines, a suspension of the flask's monolayer is used to set up the necessary number of 24-well tissue culture plates. One plate from each cell line is used for a day's set up. When specimens are submitted for virus isolation, they are inoculated into designated wells on each cell line. Daily the trays of plates are observed microscopically for cytopathic effects (CPE).

Advantages of Multi-well virus isolation:

- The low cost and relative efficiency of the technique allows for a wide variety of cell lines to be utilized. Currently MHDL uses:
 - Human Foreskin (HFS)
 - Human Lung (MRC-5)
 - Epidermoid Carcinoma, Larynx (HEp-2)
 - Rhesus Monkey Kidney, primary (RMK)
 - Buffalo Green Monkey Kidney (BGMK)
 - Adenocarcinoma, colon (Caco-2)
 - Madin-Darby Canine Kidney (MDCK) (used during influenza season)
 - Rhabdomyosarcoma (RD) (used during enterovirus season)
- Because each cell line varies in sensitivity to individual viruses, multiple viruses may be isolated. An example would be rhinovirus and adenovirus isolated on the same patient sample
- The wide variety of cell lines allows for the detection of viruses from unusual samples, for example the isolation of varicella-zoster virus, CMV or adenovirus from genital swabs
- The variety of cell lines also allows for the detection of unusual viruses, for example the detection of the first parechovirus type-3 in the United States from Sudden Unusual Infant Death (SUID) cases or the detection of a cluster of enterovirus-68 (EV68) cases during 2009 Influenza pandemic
- Overlapping sensitivity of cell lines may increase sensitivity for virus isolation. For example, Influenza A may be detected on Caco-2 cells when it did not grow on RMK or MDCK cells
- Fluorescent antibody stain confirmation can be performed directly on the plate

The MHD laboratory is proud to continue a tradition of excellence for reference virus culture and partnerships with local, state laboratories, and weekly influenza laboratory surveillance data to CDC and WHO.

MHDL PFGE for Cluster Investigations in the Milwaukee Area 2010

Note: Same organism isolated from a patient within 90 days was not fingerprinted by PFGE .

Pathogen	Clinical isolates received	Clinical isolates fingerprinted by PFGE	Clusters detected by PFGE	Isolates part of cluster	Clusters linked to outbreaks
<i>E. coli</i> 0157:H7	0	0	0	0	0
Non 0157 STEC	0	0	0	0	0
<i>Plesiomonas shigelloides</i> *	5	5	0	0	0
<i>Salmonella</i> ser Typhimurium/ Monophasic	12	12	1	3	1
<i>Salmonella</i> ser Enteritidis	18	16	4	14	1
All other <i>Salmonella</i>	53	47	5	13	2
<i>Shigella sonnei</i>	5	5	1	2	0
Other <i>Shigella</i>	8	7	0	0	0
Totals	96	87	11	32	4

*Special Project: Rule-out possible *Plesiomonas shigelloides* cluster post-severe July 2010 flash flooding in Milwaukee area.

Baildon, Braenderup, Enteritidis, Gaminara, Monophasic, Newport, 6,7:d:-

Milwaukee Health Department is currently certified by CDC to perform *Campylobacter jejuni*, *E. coli* 0157:H7, *Salmonella*, *Shigella* and *Vibrio cholerae* PFGE gel and analysis

Laboratory Confirmed Influenza by Week: City of Milwaukee Public Health Laboratory					
October 1,2010 – February 24, 2011			Positives by Type		
Week Ending	No. Tested	No. Pos.	2009 H1N1	A/H3	B
10/01/10 to 01/01/11	59	0	0	0	0
01/08/11	0	0	0	0	0
01/15/11	7	2	2	0	0
01/22/11	4	1	1	0	0
01/29/11	10	5	2	2	1
02/05/11	32	28	22	3	3
02/12/11	42	16	13	3	0
02/19/11	17	6	5	0	1
thru 2/24/2011	9	4	3	0	1
Total	180	62	48	8	6

SUMMARY OF CONFIRMED INFECTIONS

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The February 2011 issue presents the laboratory diagnosis of some of the infectious diseases and the reference microbiology work done in this laboratory during January 2011 and new cases of syphilis in City of Milwaukee during January 2011.

Syphilis

Test	Total	Test	Total
RPR Reactive	1	TPPA Reactive	7
VDRL Reactive	16	Darkfield Positive	0

New Cases of Syphilis:

Stage	Number of Cases	
	January 2011	January 2010
Primary syphilis	0	1
Secondary syphilis	0	3
Early latent	0	2
Late latent	0	10
Total	0	16

Source: Wisconsin Division of Health

Gonorrhea Antimicrobial Susceptibility Testing

Number Tested	Decreased Susceptible (DS) / Resistant (R) Antibiotics			
	Ciprofloxacin	Cefixime	Ceftriaxone	Azithromycin
18	1	0	0	0

Isolates Other Than *N. gonorrhoeae*

Organism	Site	Number Isolates	Organism	Site	Number Isolates
<i>Ureaplasma urealyticum</i>	Genital	5	<i>Mycoplasma hominis</i>	Genital	0

Enteric Parasites Identified

Age	Sex	Parasite
23	M	<i>Blastocystis hominis</i>
29	M	<i>Blastocystis hominis</i>
41	F	<i>Blastocystis hominis</i>
31	M	<i>Endolimax nana</i>

Mycobacterial Infections

Age	Sex	Test Results			Identification
		Sputum Smear	Culture	DNA Probe	
36	M	-	+	ND	<i>M. xenopi</i>
25	F	-	+	ND	<i>M. xenopi</i>

Reference Cultures

Age	Sex	Source	Identification
87	M	Pleural fluid	<i>Bacillus</i> species, NOT <i>Bacillus anthracis</i>
23	F	Cervix	<i>Neisseria gonorrhoeae</i>
27	M	Urethra	<i>Neisseria gonorrhoeae</i>
20	M	Rectum/Throat	<i>Neisseria meningitidis</i>
61	F	Urine	<i>Salmonella</i> Braenderup
56	F	Stool	<i>Salmonella</i> Brandenburg
80	F	Urine	<i>Salmonella</i> Cubana
33	M	Stool	<i>Salmonella</i> Enteritidis
2m	F	Stool	<i>Salmonella</i> Typhimurium

Virus Isolations from Clinical Specimens

Age	Sex	Source	Symptoms	Agent
9	F	NP	Influenza A confirmation	2009 influenza A H1N1
30	M	NP	Influenza A confirmation	2009 influenza A H1N1
21	F	NP	Influenza A confirmation	2009 influenza A H1N1
28	F	NP	Influenza A confirmation	2009 influenza A H1N1
19	F	Throat	Fever, headache, cough, sore throat	2009 influenza A H1N1
22	F	NP	Autopsy	2009 influenza A H1N1
19	F	Throat and NP	Fever, headache, cough, sore throat	2009 influenza A H1N1
19	F	Throat	Fever, cough, sore throat	2009 influenza A H1N1
25	F	Throat and NP	ARD, cough, sore throat	2009 influenza A H1N1
19	F	Throat and NP	ARD, cough, sore throat	2009 influenza A H1N1
21	F	Throat	Fever, headache, cough	2009 influenza A H1N1

21	F	Throat and NP	Fever, headache, cough	2009 influenza A H1N1
24	M	Throat and NP	Fever, headache, nausea, sore throat	2009 influenza A H1N1
20	F	Throat	Fever, headache, cough, sore throat	2009 influenza A H1N1
18	M	Throat	Fever, cough	2009 influenza A H1N1
20	F	Throat	Fever, headache, cough, sore throat	2009 influenza A H1N1
19	F	Throat and NP	Fever, cough, myalgia	2009 influenza A H1N1
22	F	Throat	Fever, headache, cough	2009 influenza A H1N1
21	F	Throat	Fever, ARD, URI, cough, sore throat	2009 influenza A H1N1
19	F	Throat	Fever, headache, cough, sore throat	2009 influenza A H1N1
29	M	Throat	Fever, headache, cough, sore throat	Influenza A (H3)
39	M	NP	Influenza A confirmation	Influenza A (H3)
40	M	NP	Influenza A confirmation	Influenza A (H3)
25	M	Throat and NP	Fever, cough	Influenza B
25	F	Throat and NP	Fever, cough	Influenza B
28	F	Throat	Fever, cough	Influenza B
22	M	Throat	Fever, URI, cough, sore throat	Influenza B
18	M	Throat	Fever, URI, cough, sore throat	Adenovirus
22	F	Throat and NP	Headache, ARD, cough, sore throat, body aches	Coronavirus
5 wk	M	NP	Autopsy	Coronavirus
28	M	Throat and NP	R/O influenza	Coronavirus
21	M	Throat	Fever, headache, sore throat, ILI	Herpes Simplex Virus type-1

Influenza Real-time RT-PCR Testing

Samples Tested	2009 Influenza A (H1N1) Positive	Influenza A (H3) Positive	Influenza B Positive
40	20	3	3

Herpes Simplex Virus Isolations

Agent	Number of Isolates
Herpes Simplex type 1	8
Herpes Simplex type 2	7

Molecular Amplification and PCR

Agent	Method	Tested	Positive	% Positive
Norovirus	RT-PCR	2	0	0%
<i>Bordetella pertussis/parapertussis</i>	RT-PCR	3	0	0%
<i>Chlamydia trachomatis</i>	ProbeTec	435	67	15.4%
<i>Neisseria gonorrhoeae</i>	ProbeTec/GenProbe	505	24	4.8%

Respiratory Virus Surveillance:

Respiratory Virus Panel Test Results		
Virus	Positives	Percent
Human Rhinovirus (HRV)	11	7.6%
Parainfluenza virus 2 (PIV2)	4	2.8%
Parainfluenza virus 3 (PIV3)	1	0.7%
Adenovirus Type E (Adeno E)	3	2.1%
Coronavirus OC43	6	4.2%
Coronavirus NL63	2	1.4%
Respiratory Syncytial virus (RSV)	2	1.4%

Samples tested: 144 (Sept. 1, 2010 – February 8, 2011)